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Agricultural Research

Inside a Cell: Plant Biotech at Work

FORUM

A Milestone for Agricultural Research *Magazine*

This month marks the beginning of the fifth decade of continuous publishing of *Agricultural Research* magazine. The prototype edition rolled off press in January 1953, just 2 months after official announcement of the creation of the Agricultural Research Service.

From its inception, *AR* magazine has endeavored:

"to furnish latest research findings to those who work directly with farmers, processors, marketing agencies, and the general public; and

to provide progress reports to Federal and State research workers, research organizations, and Members of Congress."

Today, *Agricultural Research*'s staff still pursues those original goals while also aiming to reach even more and diverse information users. Members of the news media, educators, consumers, environmentalists, and other groups with special interest in agriculture look to the magazine for regular updates on ARS research.

Through all of the changes that have occurred in ARS during the past four decades—the adding or subtracting of various official responsibilities, expanding and shrinking budgets, priority shifts, and internal and external reorganizations—*Agricultural Research* has remained thoroughly committed to its public-information mission.

This constancy has been maintained in the face of many exigencies and changes also occurring within the magazine's sphere. Under the direction of 10 different editors, *AR* magazine has published over 6,000 stories about ARS projects—by any measure, a powerful lot of technology transfer!

To browse back issues is to sense the shifting societal problems and concerns that ARS research has been intent on addressing. For example, projects reported in 1953 included stories on heat pumps for the home and farm, preserving market quality in fresh fruits and vegetables, and improving production of clinical dextran—vital as a blood-plasma supplement for wounded U.S. soldiers in Korea.

By 1963, stories were touching on world population growth and meeting projected food needs. Efforts to keep U.S. farm communities and rural areas prosperous were also prominent. And ARS utilization research on new agriculturally based products and processes was reported to be finding many applications in USDA's Rural Areas Development program.

Early 1963 saw ARS scientists pursuing about 400 projects on expanded uses for agricultural materials. Roughly half the projects were directed toward industrial applications; the rest, toward new or improved food and feed products.

Among the many mainstream contemporary food products developed from those and other ARS utilization research efforts are frozen concentrated orange juice, powdered dry (and now, frozen concentrated) milk, instant mashed potato flakes, and freeze-dried and explosion-puffed mushrooms, berries, and other fruits and vegetables.

1973 saw stories on the recovery and reuse of food and feed processing byproducts and waste. ARS focused tightly on the safe application, use, and disposal of pesticides. And on cleaning polluted water and making positive use of sewage sludge for land improvement.

The steady stream of new or improved consumer products continued, too—with products such as soft drinks and candies enriched with protein powder, beef specially bred to reduce saturated fats, and milk products for lactose-intolerant consumers.

1983 found ARS deeply involved in natural resource research, so six consecutive issues of the magazine featured soil and water conservation.

Outstanding accomplishments noted then of the agency's first three decades included: determination (with Cornell University) of the molecular structure of tRNA; development of longer lasting, wash-and-wear, durable press, soil- and weather-resistant, and flame-retardant cotton fabrics; custom-tailored plants with important new agronomic and quality features; an innovative nonchemical technique for the control of insect pests by sterile fly release; and refinement of vaccines to control costly hoof and mouth disease in livestock and Marek's disease in poultry.

When the Department of Agriculture completed its first century of service in 1962, there was speculation about the role ARS would likely play in meeting the agricultural challenges of the year 2000 and beyond.

Now, that once remote future is hard upon us. ARS' national program leaders are already planning for future research needs, up to the year 2030.

The agency hopes to remove impediments to achieving adequacy of both quantity and quality of food, feed, and fiber for the world. And to accomplish this without compromising the vigor of U.S. agriculture or the precious natural research base on which it all depends.

During coming months, staff writers will be covering 122 research locations where more than 2,200 scientists are pursuing nearly 1,500 major ARS projects. They run a broad gamut—from low-tech, practical projects, to the highly complex and theoretical.

In this issue, for example, we look at the ever-unfolding field of biotechnology. Among many things, ARS genetic engineering research promises more nematode-resistant soybeans and bruise-resistant potatoes, while induced genetic variation offers more plentiful supplies of a tasty peach.

We at *Agricultural Research* look forward to continuing to serve as a lens for viewing ARS scientists' steady progress toward solving the food and agricultural problems of today—and, tomorrow.

Linda R. McElreath
Associate Editor

Agricultural Research

Cover: This electron micrograph shows some of the components in a soybean seed cell. For better visibility, computer processing has colored seed storage proteins purple and stored oil yellow. Red areas are cellular compartments where synthesis of the stored protein and oil occurs.

Electron micrograph by Eliot Herman, ARS. Color enhancement by Terry Yoo, Science and Technology Center for Computer Graphics and Scientific Visualization at the University of North Carolina. (K5044-1)

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SCOTT BAUER

Ingenious Vehicles

Taking Apart and Rebuilding Plant Genes

Plants constantly struggle as they sprout from seed, grow, mature, and bear fruit and new seed. But no sign of this struggle is visible when a friend hands you the perfect home-grown tomato or you drive past the unblemished green of a young soybean field.

That's because these delights are tricks of nature. But how, if not by magic, do plants assemble a tomato or a soybean seed?

They do it through the biochemical traffic in and out of the cells, the plant's living engines, piloted by genes.

Genes direct cells to build proteins, hormones, toxins, and other chemicals. With these raw materials, cells create and ship energy, get rid of waste and invaders, and grow the cell and tissue structures the plant needs to survive, grow, and mature.

Today, scientists at the Agricultural Research Service are making plants reveal more of their genetic instructions. Still, only a tiny fraction of genes have been inventoried.

Technician Christine Berry checks on futuristic peach and apple "orchards." Each dish holds tiny experimental trees grown from lab-cultured cells to which researchers have given new genes. (K5011-19)

At the Plant Molecular Biology Laboratory (PMBL) in Beltsville, Maryland, the scientists are also redesigning genes: in soybeans, tomatoes, peaches, rice, potatoes, sugar beets, and other crops.

The eventual payoffs could be healthier plants, more economical farming, and more nutritious food on the table.

The red brick laboratory building is a plain-looking vessel for the scientific enterprises simmering inside, with 10 full-time ARS researchers along with support scientists, research associates, technicians, and collaborators from both the University of Maryland a few miles away—and faraway lands such as Israel and South Korea.

Genetic Cartography

PMBL geneticist Thomas Devine and several colleagues are looking very closely at genes in the soybean plant.

An $11 billion crop in the United States in 1991, this plant supplies two-thirds of our oil for food uses. The oil also has dozens of industrial uses, and the seed's high-protein meal sustains people and livestock around the world.

So the job is not to put the soybean on the map. Instead, Devine is helping put a genetic map on the soybean. To visualize a gene map and how genes act, it helps to know a little about railroads and, as you'll see, music and poetry.

On a map of a rail line, you can locate stations along the way and find the distance between them. But in a cell's nucleus, the stations—the genes—are bunched up next to each other along molecular rail lines called chromosomes.

Imagine two giants picking up either end of a railroad line and twisting the tracks in opposite directions—with the rails staying spiked to the railroad ties.

That gives you a fair image of the double helix—the molecular structure of chromosomal DNA, or deoxyribonucleic acid. Scientists discovered DNA's structure 30 years ago, although they began unmasking its logic of inheritance decades earlier.

Linkage: The Bond That Ties Inheritance

You can mark rail distance in miles, and Devine and other scientists can measure nucleic acids by counting their components—individual nucleotides.

But, he explains, scientists map the proximity of two genes by what really

More than 300 ARS and university scientists around the country are participating in research on plant genome mapping.

counts: the likelihood of both being passed on together to offspring. In soybeans, for example, a black seed coat is commonly inherited in tandem with resistance to nematodes.

The higher the probability of two traits being inherited, the closer the linkage of their genes. Closely linked genes are near each other on a chromosome, and scientists use a crucial bit of logic to expand the known network of genetic linkage. "If one gene is closely linked to each of two other genes, those two are linked to each other," Devine says.

Gene linkage makes for more practical breeding strategies. With a complete gene map, Devine says, "a breeder could estimate how many progeny would be needed to grow from parent plants to get, say, 50 test plants with a desired combination of genes."

SCOTT BAUER

Each soybean plant is one roll of nature's genetic dice. To find gene linkage—the probability of a given plant inheriting two different genes—technician Shannon Fox and geneticist Thomas Devine take and compare measurements from hundreds of plants. (K5000-20)

His efforts are part of an extensive USDA research program on plant genome mapping, coordinated by the Agricultural Research Service. More than 300 ARS and university scientists around the country are participating. Devine collaborates on soybean mapping with Beltsville colleagues and scientists at two other ARS locations and six universities.

Two gene maps hang on a wall in Devine's office. One is a rudimentary classical map of 19 linkage groups developed over the decades by plant breeders and geneticists. The other map, still evolving, integrates classical linkages with those being found through new molecular approaches.

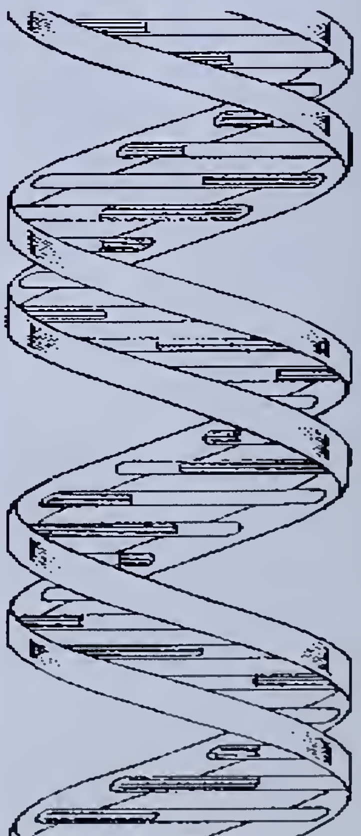
With classical methods, it took Devine 8 years to map a gene that makes soybean stems grow in a flattened, almost inside-out manner called fasciation. "Now," he says, "we can use molecular probes to look at hundreds of points along the chromosomes and examine tens of thousands of linkage possibilities. This makes gene mapping 4 to 5 times faster."

A molecular probe is a specific—usually unknown—sequence of nucleotides. With a variety of tests, scientists can often find whether and where all or part of a sequence occurs in a plant's chromosomes.

"Integrating the classical and molecular maps will yield a comprehensive map of agronomically impor-

Anatomy of the Genetic Railroad

Chemically, genes are nucleic acids—the longest living molecules, made of linked units called nucleotides. A sugar, a phosphate, and a base—adenine, guanine, cytosine, or thymine—make up a single nucleotide. Along the genetic "rails," phosphates alternate with sugars. Each sugar is chemically "spiked" to a base. An adenine base at one end of each "railroad tie" always finds thymine at the other end. Similarly, guanine complements cytosine. A nucleotide's base defines its characteristic identity, and the sequences of the four bases form each nucleic acid's unique structure.



tant genes. The molecular approach is a bridge to that map," Devine says.

On his molecular map, linked genes have cryptic names—like Rhg4, BLT24, and BLT65. Someday, the linkage of these genes by the lab's researchers could—for starters—help U.S. soybean growers overcome a microscopic, wormlike pest that carries a $250 million annual price tag.

Breeders have known that the Rhg4 gene enables the plant to resist the root-damaging soybean cyst nematode. But Devine and PMBL colleagues found that this gene is closely straddled by the other two—BLT24 and BLT65.

"This discovery," Devine says, "could lead to isolating the Rhg4 gene and transferring it into susceptible plants and varieties, making them resistant to nematode infestation."

Other scientists at the lab are finding that the two straddling genes could also have payoffs of their own. Eliot Herman found that the BLT24 gene encodes a protein made only in cells of developing seeds.

Benjamin Matthews, who cloned the other straddler, BLT65, says it makes an enzyme, aspartokinase-homoserine dehydrogenase, important in producing the amino acids lysine, threonine, and methionine. Matthews, an ARS plant biochemist, adds that "Cloning this gene could give us a new strategy for improving nutrition, not only in soybeans but also in other crop plants."

Using molecular probes, Matthews, Devine, and other researchers—Gordon Lark of the University of Utah and Reid Palmer of ARS in Ames, Iowa—have added about 200 new gene "stations" to soybean's molecular map, including 25 new linkage groups. They also found "station addresses" on soybean chromosomes for traits tied to the plant's height, leaf area, seed oil, maturity, and susceptibility to lodging.

"Once we find a gene's linkages—particularly with genes we know are tied to important traits," Matthews

KEITH WELLER

Plant physiologist Eliot Herman studies the formation of protein and oil storage compartments in the seeds and leaves of plants such as soybeans and tobacco. (K3119-2)

says, "we can home in on the genes that breeders and genetic engineers may want to exploit to improve a plant."

Fueling the Amino-Acid Machines

"We don't yet know enough," Matthews says, "about how cells perform some functions"—such as how leaf chloroplasts use carbon and nitrogen to build amino acids they ship out to nourish and fatten seeds.

In 1991, Matthews and former research associate Gregory Wadsworth found that soybean seedlings make five different forms of an enzyme, aspartate aminotransferase (AAT). AAT helps the plant control its balance of carbon and nitrogen, but "each form of ATT may have a unique role," Matthews says.

Recently, the scientists cloned a gene that produces one of the AAT enzymes. When they inserted the gene into bacteria, the microbes obediently cranked out the chloroplast form of AAT.

"This not only is a way to produce large quantities of the enzyme," Matthews says. "It also lets us alter

Genetic Sheet Music for the Cellular Orchestra

When Lowell Owens describes the modified cecropin gene he inserted into plant cells, it's clearer why such work is called genetic "engineering."

Cells, after all, are engines: They capture energy in various forms and convert it into different forms. An orchestra is also an engine: Its players take in air and send out beautiful music, if they follow the musical score.

The genes of DNA are the cell's book of molecular composition. By making copies of itself called RNA, a gene prints the appropriate sheet music and the cell plays on cue.

Owens wants the cecropin gene's music to enable plant cells to kill bacterial cells—destroying their membranes so the bacteria can't multiply and clog a plant's water tubes or attack and destroy the plants' own cells.

The gene Owens works with has three sequences of nucleotides: a promoter to turn on the RNA copier, a coding sequence to make the bacteria-killing compound, and a third sequence to ensure that the compound goes outside the cell—where the bacteria are.

To work, the cecropin gene must first be turned on by a switch—a promoter. Gene engineers use promoters to borrow one organism's ability to respond to an event, so that another organism can respond—perhaps differently—to the same event.

Typically, Owens says, such an event is one or more of a huge number of specific conditions—such as heat or cold, wounding by insects or pathogens, low or high levels of sunlight, or the plant's developmental stage.

But unlike event-specific switches, the novel cecropin promoter "sings" almost nonstop. It's a piece of DNA from cauliflower mosaic virus. In the virus, its constant task is to multiply the virus particles. But for Owens' research, the promoter was snipped from that role.

Instead, its job is to nag the cecropin gene's coding sequence into continuously writing the nucleic-acid score the cell must follow to assemble choruses of cecropin.

The third sequence serves as the molecular finale. It modifies cecropin's biochemical tune so that, rather than hold on to the protein, the cell secretes it. Circulating through intercellular space around the plant's water tubes, cecropin can attack bacteria. If Owens and colleagues can get this strategy to play well in the lab—and then in greenhouse and outdoor tests—it could be welcome music to the ears of growers.

the sequence of the AAT gene and use the bacteria to produce an altered and more efficient enzyme and, ultimately, to boost the soybean's nitrogen use and yield."

Matthews is also working to raise soybean's nutritional value. For this he turned to the common carrot. "It's relatively easy to put new genes into carrot cells and regenerate whole plants," he explains. "With soybeans, regeneration technology isn't quite there yet."

His aim is to raise soybeans' production of methionine, an amino acid soybeans currently make little of. More methionine would give soy meal higher nutrient value for animal feeds—and for foods people eat.

First, Matthews and colleagues isolated a carrot protein that acts to make two different enzymes during its

SCOTT BAUER

Plant pathologist Peter Ueng examines young wheat plants for telltale blotches of the *Septoria* fungus. (K5006-9)

synthesis of several amino acids. Later, they isolated and cloned the gene governing this dual-purpose protein.

Matthews and former PMBL research associate Jane Weisemann are patenting the gene and its use. "We want to see if we can alter this carrot gene to regulate the amino acid content in plants—in particular, lysine, homoserine, threonine, isoleucine, or methionine," he says. That could lead to better balance of amino acids in crops: more methionine in soybeans and more lysine in rice, for example.

Gene-Fest for Tomorrow's Champion Crops

Gene mappers aren't interested only in soybeans—or only in plant genes, for that matter. In searching for ways to give crop plants new genes to ward off disease, PMBL scientists are also examining genes from fungi, moths, and viruses.

Peter Ueng, a plant pathologist, wants to give wheat a better chance of surviving *Septoria* fungi. As the fungi invade and destroy cells, they mar leaves and outer seed husks (glumes) with ugly brown blotches.

More common in the humid Southeast, leaf and glume blotch can cause a 10- to 40-percent drop in yield, says Ueng.

But on some wheat leaves, the blotches have yellow halos, a sign that the plant's cells are putting up a molecular struggle to halt or slow the fungus. Now, Ueng and university colleagues have narrowed down which genes enable cells in the haloed areas to fight back.

"Our ultimate goal is to reduce yield loss and the farmer's need for fungicides by giving wheat new, powerful genes to stop the disease," Ueng says.

But the first big advance, he adds, will be an ability to forecast the virulence of new strains of *Septoria* that regularly show up. Ueng found that the fungus has an unusually large natural genetic variation. By comparing the virulence genes of known and emerging strains, he says, scientists could give farmers an early alert.

Ueng's co-researchers are with Cornell University and Purdue University; such collaboration is the PMBL's hallmark. Since January 1991, the lab's scientists reported research findings with 75 scientists at 18 ARS labs, 20 U.S. universities, and institutions in Belgium, Canada, Egypt, France, India, Israel, Italy, Qatar, South Korea, and Yugoslavia.

Down the stairs from Ueng's office, plant physiologist Lowell Owens directs and collaborates on several studies to give novel genetic defenses against bacterial wilts and rots to sugar beets and other crops. His cooperators include former research associate Russell Nordeen and scientists from Beltsville's Livestock Insects and Vegetable laboratories, the University of Alaska, and Louisiana State University.

Moth Genes Fly Bacterial Relief Missions

Pseudomonas bacteria are among Owens' chief targets. Infection starts when a root, shoving its way through soil, scrapes itself as it creeps past a sharp particle of sand. That can allow bacteria to invade and set up shop in the plant's xylem tubes, which carry water to stems and leaves. As the bacteria multiply, they make a gum that clogs the tubes. Leaves—and soon the plant—wilt and die.

In recent years, scientists looking for natural antibiotics have been testing genes from not only plants but also fungi, bacteria, and moths. A gene in the giant silk moth, for example, enables the insect to make cecropin, a small protein that fights bacterial infection. Owens has inserted a modified cecropin gene into tobacco and is testing to see if it protects the plants from wilt caused by *Pseudomonas solanocearum* bacteria. [See box on page 8.]

SCOTT BAUER

Armed with a device similar to a paper hole punch, geneticist Ann Smigocki collects leaf disks for tests aimed at learning how a genetically engineered relative of tobacco responds after overproducing the cytokinin hormone. (K5009-10)

Earlier, Owens collaborated with Steven Sinden of the Beltsville Vegetable Laboratory to test the feasibility of protecting potatoes from soft rot with a cecropin gene or a gene from chickens that makes an antibiotic known as lysozyme. In tests so far, lysozyme appears to have the edge.

Potatoes with cecropin and lysozyme genes have been tested in outdoor trials coordinated by ARS scientist William Belknap in Albany, California. Some results look promising, but they're far from conclusive.

An antibiotic could be worse than a disease if a plant got too high a dose, says Owens. But he, Sinden, and Nordeen found that cecropin is toxic to nine bacteria at levels far below those at which it harms protoplast cells of soybeans, sugar beets, and sweetpotatoes. However, tomatoes and some

potato varieties are more sensitive, so making cecropin work without harming these plants may be difficult.

Harmonizing With Hormones

To know if a new gene can benefit plants, PMBL scientists first have to make cells reveal more about how their existing genes run the business. To harmonize the plant's chores of producing and storing its food and growing to maturity, cells generate a key hormone called cytokinin, says geneticist Ann Smigocki.

"Cytokinin has a broad role in the normal growth and development of a plant. It carries out this role partly by controlling the expression of many plant genes," says Smigocki.

To make cytokinin reveal its genetic tinkering, she has genetically engineered several plants including tobacco, tomato, peach, and others. She turns cytokinin on and off by equipping these plants with a different cytokinin gene—one whose DNA switch, or promoter, she can control.

Some promoters originate from genes in plants; others, from insect genes. One promoter—from fruit flies—turns on one of the genes that help this insect avoid or recover from heat shock.

Smigocki hooked this promoter to a cytokinin gene and engineered the assembly into tobacco. After exposing plants to 125°F heat for an hour, she found much higher levels of several proteins. The heat-activated promoter had roused the cells to make cytokinin. Using this and other gene switches, Smigocki and research associate Scott Harding have isolated more than 200 cytokinin-affected genes, and are closely examining about 70. They want to match up the genes to the proteins whose levels rise or fall when cells crank out cytokinin.

Smigocki says identifying the proteins—and how, when, and where they act—will help reveal how to

SCOTT BAUER

What an electron microscope can't reveal, other tools can. Here, research leader Autar Mattoo (left) and research associate Tedd Elich use a two-dimensional laser densitometer to quantify variants of a key chloroplast protein on an autoradiograph. (K4986-20)

manipulate their associated genes for better yield or other desirable traits.

One promoter/cytokinin assembly she uses to track the hormone's influence may enable plants to act quickly to retard caterpillars and other attacking insects. "What we've done is get plants to overproduce cytokinin when they're being eaten," says Smigocki, who is patenting the approach. In lab and greenhouse tests, tomato hornworms ate and grew at less than half the normal rates when their diet was leaves from plants harboring the new gene.

When Cells Lose Their Grip on Light

As a growing plant struggles to cope with insects and diseases, its leaves must continue harvesting light to make food—the business of photosynthesis.

Why, then, will a perfectly healthy bean leaf turn yellow and drop off during the course of a few days? This apparently trivial event has huge implications, says plant physiologist Autar Mattoo, who is head of the PMBL.

When a leaf matures, he notes, it simultaneously stops growing and loses the ability to capture sunlight and convert the atmosphere's carbon dioxide into food. He and colleagues—Roshni Mehta of the University of Maryland and former research associate Timothy Fawcett—uncovered some of the gene-driven events responsible.

"This is taking us closer," Mattoo says, "to genetically engineering crops that can make their own food for a longer, or shorter, time during the growing season. That could lead to earlier harvests or later, larger harvests and more nutritious crops."

In studies with wheat and an aquatic plant, duckweed, the scientists used copper ions to artificially age leaves in a few days. That made it possible to measure biochemical changes in the chloroplasts—the hundreds of gene-filled foodmaking factories in each leaf cell.

Mattoo and colleagues found that the aging process yielded hordes of a type of oxygen atoms known as free radicals. These atoms-run-amok have

been associated with a breakdown in growth processes and disease defenses in humans, livestock, and other animals.

In Mattoo's test plants, the free radicals rearranged an amino acid in rubisco, a photosynthetic protein.

How important is rubisco?—By changing carbon to a form plants can use, it makes life possible on this planet. But retooling the amino acid in the test plants inactivated their rubisco.

"Dead" rubisco proteins travel to the chloroplast's membrane and are then disassembled by enzymes, Mattoo explains. Some rubisco elements—nitrogen, for example—are sent to other parts of the plant, such as seeds that are filling or leaves still on the light-grabbing side of maturity.

"The plant recycles what it can use and sends any surplus to the soil, via its roots," Mattoo says. "We still need to identify the genes and the mechanisms controlling this and other processes that shut down the chloroplast factories. Then we can see how photosynthesis is affected in plants given a variety of differently reconstructed genes."

Reducing Solar Energy Waste—and Sunburn

The shutdown of chloroplast factories isn't Mattoo's only concern. Plants have had billions of years to perfect their photosynthesis machines, but they seem to vastly underuse their energy resources.

"Plants can use only about 2 percent of the sunlight reaching their leaves," he says. And he's fingered D1—the key protein in the chloroplast's membrane—as one of the culprits. "Plants expend enormous energy," he says, "to rapidly make and then degrade this protein in sunlight—more rapidly if given extra ultraviolet light. We want to know how and why."

Earlier, the scientists found that some weed-killing chemicals bind to D1, block the movement of electrons through it, and inhibit its breakdown. Now they've found that these are three separate, not linked, properties of the D1 protein. "We are looking into this further," he says, "because we want to use bioengineering to slow D1's degradation and then analyze the plants' photosynthetic efficiency."

Last year, Mattoo and Marvin Edelman and others at Israel's Weizmann Institute of Science discovered that ultraviolet-B radiation destroys the D1 protein.

UV-B is a part of the light spectrum known to cause skin cancers in some

MARK TUCKER

A scanning electron microscope photo reveals the layer of rounded, swollen cells on a kidney bean stem where a leaf recently abscised, or separated, from the stem. (92BW1869)

people who get too much sun over a prolonged period of time. Much of the sun's UV-B is screened by Earth's natural ozone layer, but pollution has thinned the ozone by 5 to 10 percent in the past 20 years.

Mattoo says the new finding about D1 lends new significance to ozone depletion. "An increase in UV-B reaching Earth could eventually translate into less crop productivity—and less food. That's because UV-B, by destroying D1, reduces a chloroplast's efficiency in carrying out photosynthesis," he says.

He and former research associate Tedd Elich also found a change in D1 under normal light. "A phosphate molecule lodges onto one of its amino acid building blocks," Mattoo says. "This may have to happen before light can break down the protein."

The next step is to see how minor alterations to D1's gene affect a plant. "That will gradually tell us what turns this gene on and off. In other words, what tells the gene when to build and when to destroy D1 protein," Mattoo says.

This approach is sometimes tagged with the violent-sounding title of "gene bashing." But it can be compared to the editing of poetry. The verses are linked fragments of genes; the syllables are nucleotide sequences. Editing is done with probes—biochemical "blue pencils" that scientists use to delete one to thousands of nucleic acid syllables.

If editing quickens or slows the biochemical rhythms under study, scientists know they've touched—or bashed—a gene they want to know better.

Dying on the Vine: It's Natural

Remember that falling bean leaf? While Mattoo wants it to have a longer, more productive youth, molecular biologist Mark Tucker wants to know why it detached itself, or abscised.

Tucker also wants to know why flowers sometimes take a dive instead of staying on to produce fruit, and how to get ready-to-harvest crops such as apples, beans, cotton, oranges, nuts, and tomatoes to cooperate better with hand or machine harvesting.

Using a scanning electron microscope, Tucker and colleagues examined the area of cells on the abscission layer, where stem and leaf perform the delicate biochemical surgery.

Abscission does not rupture the cells, they found. Instead, the cells themselves dissolve the "glue" that had bound a single layer of stem cells to cells of the departing leaf.

Stem cells, says Tucker, team up to amputate a leaf with little or no "bleeding" of the stem's moisture or nutrients. The cells wield an enzyme, cellulase, for this surgery.

"It's been known for some 40 years that cells make cellulase and that it breaks down some component in the cell's wall," he says. "We need to find out more about how genes and hormones interact to drive this process, so we can modify the genes to control it."

Scientists already know the key hormones: Ethylene promotes abscission, and auxin retards it.

Earlier, Tucker and collaborators identified and sequenced a cellulase gene from beans. When the gene springs into action, it makes messenger ribonucleic acid, or mRNA. This mRNA acts as the cell's work order: "Make cellulase now!"

In abscised leaves, Tucker and colleagues found that cellulase mRNA occurs only in the two layers of cells on either side of the separation zone and in the vascular tissue that feeds the leaf.

Currently, he's examining tomato plants to which he gave different sets of the cellulase gene from the bean plant. The strategy is to edit the cellulase gene until it reveals its promoter, or switch.

"We would use the promoter," he says, to make a substance such as auxin. That would stop the gene from making cellulase when we don't want it made—for example, when it causes premature abscission in flowers and fruit."

He works with tomatoes because the cells are amenable to gene engineering and regrowth into whole plants. But one of his aims is to reduce flower abscission in soybeans.

Like most plants, soybeans make more flowers than necessary—as insurance. "About 70 to 80 percent of soybean flowers drop off prematurely," he says, "because of drought, insects, or other stresses. But you wouldn't want all the flowers to stay on anyway, because the plant couldn't support all the resulting seeds."

Other scientists plan to control abscission by exploiting the front end of the biochemical chain of events.

SCOTT BAUER

To understand plant aging, research assistant Roshni Mehta (left) and research associate Ning Li isolate stress- and ripening-related proteins from plants. The goal is to improve quality and postharvest storability of fruits and vegetables. (K4985-12)

The strategy is to alter a soybean gene for an enzyme called ACC synthase. This enzyme is the key controller over the hormone ethylene, says Mattoo. Ethylene stimulates cells in the abscission zone to make cellulase.

"We cloned this soybean gene and inserted it into bacteria, which then produced the enzyme," says Mattoo, who did the work with PMBL research associate Ning Li and University of Maryland graduate student Derong Liu. "Eventually, we want to genetically engineer soybean plants to shut down ACC synthase. That would stop ethylene, cellulase, and as a consequence, abscission."

Cell Suitcases Stuffed With Oil, Protein

Soybean plants may be too free with their flowers. But while this means fewer seeds for growers to harvest, it's part of the plant's reproductive strategy. To carry out the strategy, chloroplasts in leaves prepare and ship the ingredients, but what do seed cells do with them?

By finding answers, PMBL scientists hope to engineer a soybean to serve the grower's purpose, not just its own.

That means they must—like auditors—make seed cells answer for the resources they accumulate and spend.

Plant physiologist Eliot Herman and colleagues delve into the seed cell to find out how its genes control machinery for churning out oil and proteins and storing these for later use when the planted seed germinates.

In cells of a developing seed, Herman explains, four key proteins form molecules of oil into thousands of microscopic droplets. Each droplet is wrapped in a membrane made of these proteins and phosphorus-rich lipids. No membrane means no droplet.

Herman and research associates Deborah Loer, Andy Kalinski, and Daniel Rowley are taking apart genes that tell cells to make the membrane proteins. "We are trying to find out how these genes are turned on and off in response, perhaps, to environmental stresses such as drought, heat, and inadequate nitrogen," he says.

The scientists identified and cloned two genes responsible for the most common membrane protein, 24 kDa oleosin. "We're using the clones," Herman says, "to find out how the cell controls the accumulation of oil bodies. We may be able to alter them to force the seed cells to make less oil and more protein for meal."

That protein is packaged in cell vacuoles, or large fluid-filled reaction chambers. They take in, process, store, and digest nutrients and expel waste and excess water.

Typical cells have one vacuole. Enclosed by a membrane, it occupies most of the volume of the cell. But in seed cells, the vacuole divides into thousands of small, protein-filled units. These and the oil droplets turn the cell into a stuffed suitcase of nutrients.

Having many small vacuoles means a vastly larger area of vacuole membrane, so a germinating seed can get at lots of protein in a hurry. "Subdividing the vacuole is the seed's way of chewing its food so it will be easier to digest later, when the seed sprouts," Herman says.

To digest stored protein, germinating seeds make yet another type of protein, thiol protease. Herman and Kalinski found that one thiol protease, P34, is made only in cells of still-developing seeds. They are now investigating whether it has a role in seed/cell protein storage.

"With the P34, oleosin, or other genes, we may be able to genetically alter the soybean plant to produce new varieties that yield less oil and more meal—or more oil and less meal. But to do that," Herman cautions, "we may have to get around a plant's habit of breaking down 'foreign' proteins. If these protein molecules are, for example, wrongly folded, the protein won't accumulate where we want it to—such as in vacuoles or oil-droplet membranes."

Folds in a protein molecule allow it to hook up to other proteins in a usable, three-dimensional manner.

To detect bad folds, cells make a special protein. Called binding protein, or BIP, it sidles up to wrongly folded proteins, perhaps triggering their breakdown. To learn how BIP works, Herman, Kalinski, and Rowley examine clones of BIP genes that govern folding of an altered storage protein from the common bean.

Why do this? The soybean industry wants to lift the crop's low sulfur content—to increase its value, especially as livestock feed. "This might be possible," Herman says, "by engineering the plants to incorporate more sulfur-containing amino acid blocks—cysteine and methionine—into proteins. But some of the bioengineered proteins are unstable. Therefore, to make it work, the folding problem will probably have to be solved."

SCOTT BAUER

More nutritious rice could come from descendants of these plants, whose ancestors were grown by Gideon Schaeffer from tissue-cultured cells specially selected for their high lysine content. (K4994-19)

Well-Rounded Meals—From Mutant Rice

Rice plants, like soybeans and other plants, pack a lot of protein into the seed—a high-energy jolt to start the next generation of plants. But Gideon Schaeffer's new rice—started from cells in the lab—could do a better job nourishing the next generation of people.

Last fall, he released the first five breeding lines of rice with protein that's high in lysine, an amino acid essential for human health. Commercial rice—like all major cereals—is low in lysine, and extra lysine would make rice a more nutritionally balanced food.

Schaeffer's high-lysine rice has slightly more total protein and 15 percent more lysine than parent plants from a commercial variety, Calrose 76.

"We first came up with high-lysine rice over a decade ago. Now we've stabilized the trait and defined some of the biochemistry so breeders can develop high-lysine commercial varieties," says Schaeffer, a plant physiologist. New varieties could be available to growers in a few years.

Rice is relatively minor in diets in developed countries, except Japan. But, he says, "high-lysine rice could improve the diet of people in developing countries, such as those in Africa and Asia where rice is the main—sometimes nearly the only—protein source." The most interest so far is from Chile, India, and China.

"Almost all rice," he adds, "is consumed in the same countries where it's grown." In 1990, only 3 percent—15 million metric tons—of the world's rice went on the international market. Two-thirds of the exports came from three countries: Thailand, Vietnam, and the United States, with the last accounting for about 3 million tons.

"Traditionally," he points out, "breeders in this country have been most interested in yield—that's the producers' first requirement. But high-lysine grain, with yield and other qualities similar to commercial varieties, should be worth more to grow."

To develop his high-lysine rice, Schaeffer used tissue culture techniques to select rice cells with a genetic mutation that kept them making lysine far longer. He did this by challenging rice embryo cells with

TIM McCABE

Peach lover and plant physiologist Freddi Hammerschlag shows off harvest from disease-resistant trees that started life as tissue-cultured cells in her lab. (K2764-3)

high doses of lysine and similar compounds, which ordinarily would kill them. From surviving mutant cells he grew whole plants.

High-lysine seed looks chalkier—more opaque—than regular rice, which is quite translucent. Since past breeding didn't favor chalkiness, it may also have lowered lysine content, Schaeffer notes.

"Continuing studies of rice cells in tissue culture," he says, "give us an efficient and powerful system to examine how cells make amino acids and synthesize and transport protein."

To make a protein molecule, a seed cell has to assemble a jigsaw puzzle. Each piece is a series of amino acids whose order is fixed by the cell's genetic machinery. Genes also command the folding of each amino acid series into the correct shape for assembling proteins to be used in the cell or stored elsewhere.

Schaeffer's rice plants work longer at making lysine-rich proteins. He is identifying the messenger RNA's that are active when cells do this and is getting very close to identifying the altered genes. "When we can do that," he says, "we will try genetically engineering rice and other crops for higher lysine."

High-lysine cells in culture may have industrial applications. "Some of the high-lysine cell lines spit out twice as much protein into the cell growth medium, and the protein is more water soluble," Schaeffer notes. He says these traits could boost production of pharmaceuticals and other high-value substances.

Peach Orchards With a Cultured Pedigree

Cell culture has been carried almost to an art form by plant physiologist Freddi Hammerschlag. Her work shows how scientists are putting together all the pieces to build better plants.

Look in almost any peach orchard in the Southeast, she says, and you'll find a potential home for bacterial spot

disease. Leaves and fruit of every peach variety are to some degree vulnerable.

The bacterium, *Xanthomonas campestris,* emits a toxin or toxins that attack cell membranes. If the cell can't put up a strong defense to neutralize the toxins, its membrane weakens. Bacteria invade and multiply; the cell dies.

For the last decade, Hammerschlag has been developing peach trees that put up a stronger cellular defense. And it's clear she's an ardent peach lover, when she shows off her hundreds of peach trees—living in a refrigerator in the building's basement. There, covered glass dishes brim with tiny green trees smaller than alfalfa sprouts.

Hammerschlag grows such trees—and their full-size orchard-dwelling offspring—from clumps of cells. "I develop technology, not new plant varieties," she says. "We want people to use the technology to make healthier peach trees and higher quality peaches."

That's what she has in mind for Redhaven and Sunhigh, two commercial varieties. "Sunhighs are the sweetest, juiciest peaches I've ever eaten," she says. "But many growers won't have anything to do with this variety because it's highly susceptible to bacterial spot. Redhaven is only moderately susceptible."

Hammerschlag improved the resistance in trees derived from both varieties by inducing changes in the genes in peach cells, then finding and testing potentially useful changes.

"Genetic variation is what we look for," Hammerschlag says, and she triggers even more by using various hormones and nutrients to culture cells. "We can also determine," she adds, "that only cells with a certain type of genetic variation will survive the tissue culture cycle." How? By adding to the culture medium the toxins produced by the bacterium to help it develop and spread disease.

The only cells that survive have a genetic ability to withstand the toxins. The approach, called in vitro selection, is also how Gideon Schaeffer developed high-lysine rice.

With cooperators in North Carolina, Hammerschlag now has the first evidence that her strategy works against bacterial spot in an orchard.

She's pursuing the same strategy with apples and another bacterial disease, crown rot. And she and cytokinin expert Ann Smigocki recently took tissue culture selection a step further. They accomplished a first by regenerating peach plants from embryos to which they had given a new gene for this hormone.

"Cells with the new gene produced many shoots," says Hammerschlag, "and the leaves had levels of two cytokinins that averaged over 50 times higher than leaves of unaltered plants." When grown to maturity, the trees were more compact—a potentially useful trait in high-density orchards, she notes.

Hammerschlag and Lowell Owens, meanwhile, are trying to transfer other foreign genes into peaches, including a moth gene for the cecropin protein. Can the moth protein control bacteria blamed for leaf spot and canker in peaches and fire blight in apples and pears?

Tests may reveal the answer to this question in a few years. By then, however, the lab's research team will be laying the gene railways of plants toward other new destinations.—By **Jim De Quattro,** ARS.

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Peach Trees That Fight Back

In 1987, ARS scientist Freddi Hammerschlag and David F. Ritchie and Dennis J. Werner of North Carolina State University planted more than 150 peach trees in an outdoor test for resistance to bacterial spot.

Some of the trees at the university test site were conventional Sunhigh and Redhaven grafts on standard rootstock.

Others were propagated in tissue culture from axillary buds. This latter technique, called micropropagation, yields large numbers of plants that are clones, or genetic twins, of a parent plant.

About 100 of the test trees began as immature embryos—five Sunhigh and two Redhaven—that Hammerschlag removed from seeds. She nurtured the embryos to form cell cultures and then small plants, and she cloned identical trees from each plant.

Several of the embryo-derived trees were more resistant to bacterial spot in the 1990 and 1991 field tests. The most resistant were grown from cells derived from a Redhaven embryo. Bacterial spot damaged only 13 percent of these peaches, compared with nearly half the peaches from Redhavens that hadn't undergone tissue culture.

Another set of trees came from toxin-resistant cells of a Sunhigh embryo. Fruit damage was considerable—but about one-third less than on standard Sunhighs.

Next, Hammerschlag will evaluate the offspring of these top-performing plants. "We got a lot of new information from those tests," she says. "For example, several genes apparently help the plant resist the disease. We'd like to isolate those genes so we can improve disease resistance even more."

Fine-Tuning Agricultural Inputs

Agricultural Research Service and University of Missouri scientists are helping farmers prove the benefits of prescription farming—the newest trend in managing agricultural inputs.

Prescription farming means applying inputs such as fertilizer, herbicides, and irrigation water with more precision than has generally been used. Farmers routinely apply fertilizers using an average rate based on a field's past yield and condition.

But environmental concerns are prompting both researchers and growers to look at alternative farming methods that offer good yields and profits while at the same time reducing the use of chemical inputs.

"Soils vary from point to point within a field. Depending on the variability of soil fertility, different spots in a field could require much more or less fertilizer than the average," says ARS agricultural engineer Kenneth A. Sudduth, who is based at the University of Missouri at Columbia.

Applying inputs only where needed increases production efficiency and reduces the potential for contaminating ground or surface water.

Sudduth views the prescription farming system as a three-step process.

"First, the farmer needs to collect data about soil variability within the field and pinpoint where that variability occurs. Then, the information is analyzed and used to develop a variable rate application plan. Finally, the farmer applies fertilizer or pesticide according to the plan," he says.

ARS and University of Missouri agricultural engineers are working on technology to make prescription farming systems more efficient.

Sensors Are Promising Tools

Sudduth and other ARS and Missouri researchers are developing sensors, investigating field variability, developing data management techniques, and modifying application equipment. Each of these represents a piece of the prescription farming puzzle that requires engineering research to make all the pieces fit together into an integrated, high-tech system that is easy for farmers to use.

Researchers have already made strides in developing electronic sensors for soil properties. These devices will help farmers map variations in a field more easily and with more detail than would be practical by hand-collecting soil samples.

"Even in fields where soils are similar, there can still be variations," says Sudduth. "For example, organic matter content can vary considerably, even within a soil type, and that content directly affects the amount of herbicide needed for good weed control.

"Soils with more organic matter appear darker. This observation gave

BRUCE FRITZ

Agricultural engineer Kenneth Sudduth examines a sample of grain collected from this combine's grain flow sensor. (K4911-19)

us the idea that we could scan a field with an electro-optical sensing device. Using sensor data, we can recommend a variable rate for soil-applied herbicides," says Sudduth.

Under a cooperative research and development agreement with a company in Springfield, Illinois, known as AGMED, Sudduth and ARS agricultural engineer John W. Hummel at Urbana, Illinois, developed a portable near-infrared reflectance soil sensor. The machine reads the amount of soil organic matter and moisture in less than 60 seconds.

They first tested soil samples in the laboratory and then took the machine into Illinois fields. Sudduth is now making some modifications to the sensor for improved reliability and accuracy. AGMED has a license to make and sell the machine, which has been jointly patented by the company and ARS.

BRUCE FRITZ

Engineering technicians Bill Wilson (left) and Bob Mahurin record the location where soil samples were taken, using a portable Global Positioning System receiver equipped with a bar code reader. (K4913-7)

Soil nitrate measurement is another important element in prescription farming. Hummel is at work on a sensor to measure nitrate levels for use in recommending variable rates for nitrogen applications.

Stuart Birrell, a University of Missouri agricultural engineer who is also working with Sudduth, notes that development of the sensors is lagging behind the rest of the technology needed for prescription farming. "While some of the other components of the system are already in commercial use, most sensor technology is still in the prototype stage at best."

What About Yields?

Another prescription farming project involves measuring yield variability. "Yields and profit are very important to farmers. Being able to accurately measure yield variability within the field helps the farmer decide how to apply nutrients and gives an indication of how well the prescription farming method is working," says Sudduth.

In 1991, he and University of Missouri agricultural engineer Steve Borgelt equipped a combine with a grain flow monitor that's sold only in Europe. With it, they monitored yield variability on test plots at the Missouri Management Systems Evaluation Area site near Centralia.

"We were surprised that the 1991 yields varied by as much as 50 percent within a 600-foot plot," Sudduth says.

For the 1992 harvest, the combine was equipped with a Global Positioning System (GPS) receiver to pinpoint its location in the field. GPS is a network of satellites developed and maintained by the U.S. Department of Defense. The receiver, mounted in the combine, receives signals from the satellites and pinpoints the combine's position to within 15 feet.

The researchers are also using a GPS unit after harvest, or the next spring, to locate spots in the field where soil samples are collected. The samples are then analyzed to help determine optimal fertilizer rates for the coming year. [For more on GPS, see *Agricultural Research*, February 1992, pp. 4-8.]

Missourians are known for their "show me" attitude. So, it's not surprising that Missouri farmer Bill Holmes, whose farm is located in the southern part of the state, asked ARS and University of Missouri researchers in 1991 to help automate data collec-

BRUCE FRITZ

Missouri farmer Bill Holmes (center), Kenneth Gilmore (left) of the Space Remote Sensing Center, and University of Missouri specialist Stuart Birrell examine soil fertility variations in Holmes' cropland. (K4914-2)

tion and manage data needed for the Missouri Agricultural Water Quality and Precise Application Project, which began in the fall of 1989.

Holmes is the driving force behind the project to demonstrate and apply prescription farming methods. The project includes 40 producers with 10,500 acres and several cooperators: the University of Missouri (Columbia), Missouri Department of Natural Resources, U.S. Environmental Protection Agency, and USDA's Agricultural Research Service and Soil Conservation Service (SCS).

To measure nutrient variations in their fields, Holmes and other farmers collected some 4,000 soil samples using a 330-foot-square grid.

After the University of Missouri soil lab analyzed the samples, Holmes loaded the results into a computer database along with information from a detailed SCS soil survey.

"Holmes also wanted to measure yields, and we've been able to help him do that with our instrumented combine," says Sudduth.

Preliminary analysis of the data taken in the fall of 1992 from one 160-acre field indicates yields ranged from 140 to 215 bushels of corn per acre.

The next step is to load the yield and soil data for the fields into a farm-level Geographic Information System (GIS) developed at the NASA-supported Space Remote Sensing Center in Mississippi. Birrell is helping programmers at the center incorporate advanced analysis techniques into the GIS.

The GIS is used to map and compare variability patterns in order to determine the best way to manage each field. Then, data can be directly transferred from the GIS to an automated fertilizer applicator to provide the correct amount of nutrients tailored to each area of the field.

The cooperative effort between Holmes and the researchers has been good for all. "We've been able to help Holmes implement a real world system, and at the same time we've been able to evaluate our technology and verify research findings under practical field conditions," says Sudduth.

What Happens Next?

Before prescription farming is widely adopted, farmers will need to know just how much it will help increase their yields if they switch to the new technology. To find out, ARS and University of Missouri researchers plan the ultimate test by planting side-by-side comparison strips using both average and variable rate applications of fertilizer. Crop yields, nutrient movement, and economic returns will be monitored and analyzed.

"We'd like to get an objective look at both yields and profits. The savings aren't necessarily realized from using less fertilizer or other inputs, but from getting the most yield from what is used," says Sudduth.

He adds that the prescription farming techniques available today require farmers or their custom applicators to invest a considerable amount of extra time and effort. Although research results are promising, it will likely be several years before the more integrated and easy-to-use systems attractive to a wide range of farmers are available.

Even so, researchers and progressive farmers like Bill Holmes are excited about the promise of a technology that offers increased profits and environmental protection.—By **Linda Cooke,** ARS.

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Popcorn Heads West

Popcorn farming, now done mainly in the Midwest, may increase to major proportions in the Pacific Northwest. In part, the shift could result from a casual conversation during a break at the 1988 Entomological Society of America meeting in Louisville, Kentucky.

Agricultural Research Service entomologist Richard L. Wilson of the North Central Regional Plant Introduction Station at Ames, Iowa, mentioned to another scientist that he had been screening, or testing, popcorn germplasm for resistance to corn earworm and European corn borer.

The popcorns had been left to the Plant Introduction Station by the late J.C. Eldredge, an Iowa State University popcorn breeder from 1921 to 1960.

"Of the 35 samples screened, 1 red inbred popcorn, P.I. 340856, turned out to have silks with unusually strong resistance to corn earworm feeding. But normally, popcorn in the Midwest is hardly damaged by the insect anyway," Wilson remarked to fellow entomologist Gary L. Reed. Perhaps, however, the resistance could be transferred to agronomic varieties of sweet corn that are now quite susceptible.

Reed, superintendent of the Oregon Agricultural Experiment Station's center at Hermiston, responded, "Corn earworm is our number-one popcorn pest in Oregon!"

After the meeting, Wilson returned to Ames, where he completed screening all 299 popcorn accessions in the National Plant Germplasm System collection. He then sent the Eldredge collection, plus several other of the most promising accessions, to Reed for evaluation under field conditions in Oregon.

Now, breeders are using several of those lines with varying degrees of corn earworm resistance to develop new popcorn cultivars suitable for commercial production in Oregon, Idaho, and Washington.

In Georgia field conditions, ARS entomologist Billy R. Wiseman of Tifton, also found that several of the lines resisted corn earworm and moderately resisted fall armyworm.

BRUCE FRITZ

Laboratory technician Sharon McClurg measures damage from corn earworm feeding. (K4823-1)

In laboratory studies at Athens, ARS chemist Maurice E. Snook has identified chemicals in the silks that are toxic to the earworms. The major chemical he found was maysin, with concentrations about four times that found in a Mexican corn race, Zapalote Chico, discovered a number of years ago. The research in Georgia should help scientists pinpoint resistance-conferring genes to expedite the transfer of resistance to sweet corn varieties.

Breeding insect resistance into popcorn and sweet corn is becoming an increasingly important goal as more and more pesticides for food crops are taken off the market. In the Pacific Northwest, the advent of popcorn hybrids resistant to corn earworm could accelerate a trend toward increasing acreages of popcorn grown for food. Last year, farmers in Washington and Oregon planted about 5,000 acres of edible popcorn.

Popcorn vendors may find corn grown in the Snake and Columbia River Valleys, where humidity is low, to be very desirable, says Reed. After drying naturally and uniformly in fields, it pops up fluffier and is unlikely to have many kernels that don't pop.—By **Ben Hardin,** ARS.

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Popcorn Potpourri

Americans consume 16.5 billion quarts of popped popcorn annually, according to The Popcorn Institute. Sales of unpopped popcorn grew to more than 1 billion pounds last year. Furthermore:

- About 30 percent of the popcorn is eaten outside the home—in theaters, ballparks, schools, etc.
- One cup of unbuttered air-popped popcorn provides 1.3 grams of dietary fiber and about 27 calories—up to 126 calories if lightly buttered.
- Dieters may choose popcorn for snacks to avoid "empty" calories lacking in minerals and vitamins. Popcorn has more protein, phosphorus, and iron than potato chips, ice cream cones, pretzels, or soda crackers.
- Popcorn pops because heat builds steam pressure inside the seed. This happens best at 13.5 to 14 percent moisture. Adding salt before popping toughens the popped corn.
- Archaeologists have found ears of popcorn in New Mexico nearly 5,600 years old, according to radio-carbon tests.

Salmonella Carriers Worry Scientists

It is well known that pigs infected with *Salmonella typhimurium* bacteria can be treated with antibiotics and recover.

But what worries microbiologist Paula J. Cray and immunologist Thomas J. Stabel of the National Animal Disease Center in Ames, Iowa, is that some pigs appear to recover but still carry the disease.

"Pigs can be infected but show no signs of disease. And even after careful administration of antibiotics, small pockets of the bacteria may still remain in the pig's internal organs," says Cray.

So the object of her research is to eliminate or reduce as much as possible the carrier state.

"To this end, our approach is to reduce the number of infected pigs, as well as decrease the number of bacteria carried by infected pigs. Doing either of these successfully will decrease the risk of human exposure," says Cray.

Risks can be minimized, however, by following established food safety recommendations. [See box.]

In market pigs, shipping stress appears to reactivate *Salmonella* bacteria in those pigs that are infected but show no signs of disease. Once reactivated, the organisms are shed in

SCOTT BAUER

(K4702-6)

fecal matter. Bacteria quickly spread among pigs crowded together in trucks, says Cray.

She would like to understand more about how the bacterium is passed from infected pigs to those that have never been exposed. The most significant finding in her work so far has been that pigs can become infected soon after being exposed to very low levels of the bacteria. Cray found that uninfected pigs can shed *Salmonella* bacteria within 2 days of exposure to infected pigs. And piglets can become infected after birth and before weaning, if they are nursing a sow that's shedding *Salmonella*.

"This was a surprise and it changes our approach to using new vaccines," says Cray.

She has been evaluating the effectiveness of a dozen genetically modified vaccines in which the bacteria have part of the virulent region of DNA missing. Roy Curtiss, III, of Washington University in St. Louis, Missouri, created the vaccines that Cray has tested in pigs.

"The altered vaccine strain allows an animal's immune system to develop a response, but does not overwhelm the animal and cause disease. Ultimately, the bacteria are cleared from the animal," says Cray.

"Two of these modified vaccines look promising. In tests, they didn't cause disease or weight loss in pigs receiving oral doses," says Cray.

An effective *Salmonella* vaccine is badly needed. At best, current vaccines may modify the course of the disease and the severity of symptoms, but they haven't been shown to eliminate the disease, says Cray.

The researchers have yet to determine when and how to vaccinate pigs to achieve a level of immunity that's effective against a small number of bacteria.—By **Linda Cooke,** ARS.

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Food Safety Recommendations

Salmonellosis is one of the most common types of foodborne illnesses.

Its common symptoms are nausea, vomiting, fever, and diarrhea. Most people recover fairly quickly from the illness, but it can be fatal to those with weakened immune systems. Most at risk are pregnant women, infants, the elderly, and AIDS patients.

Salmonellosis is often caused by improper storage and cooking of meats that may have been contaminated. Therefore, it is important that all food products be handled and cooked properly.

The USDA's Food Safety and Inspection Service recommends:

• Refrigerate raw meat promptly and store for no more than 3 to 5 days; or freeze, if you do not intend to use soon after purchase.

• Wash hands thoroughly, both before and after handling raw meat.

• Use a plastic, dishwasher-safe cutting board—not wood.

• Wash countertops, cutting board, and any utensils that have come in contact with raw meat with hot, soapy water.

• Don't prepare, cut, or carry raw meat on the same plate that you later use for cooked meat.

• Don't let juices from raw meat drip on other foods.

• Cook raw pork and beef to an internal temperature of 160°F at its thickest point and poultry to 185°F.

AGNOTES

Stopping Jointed Goatgrass in Wheat

Jointed goatgrass is becoming a particularly troublesome weed in winter wheat fields and has decreased yields by 25 percent or more. Commercially available herbicides that kill goatgrass also kill wheat.

Goatgrass, like winter wheat, sprouts in the fall, goes dormant in the winter, and starts growth anew in the spring. Both reach maturity by early summer. Goatgrass weed seeds that are harvested with winter wheat end up being milled with the wheat into flour. This reduces the flour's breadmaking quality and limits export opportunities—especially to our Asian markets.

"Jointed goatgrass has infested at least 5 percent and perhaps as much as 10 percent of the winter wheat acreage in Colorado," says Randy L. Anderson, an Agricultural Research Service agronomist who works at Akron, Colorado.

Washington is a particularly hard-hit state, with about 25 percent of its winter wheat acreage infested by goatgrass, reports Alex G. Ogg, Jr., ARS plant physiologist at Pullman, Washington. Ogg says weed scientists in the western half of the United States estimate that at least 3.5 million acres of winter wheat are infested with jointed goatgrass.

The biggest problem is that some growers have so many jointed goatgrass plants that the only solution available to them now is to mow their fields for hay. This produces only a third of the income they would have obtained from a grain crop.

"But harvesting for hay can be the beginning of a successful control system that includes crop rotations. Growers could plant summer-annual crops, such as corn, sorghum, or sunflowers, for 2 consecutive years," says Anderson at ARS' Central Great Plains Research Station.

Crop rotations encourage germination of goatgrass seeds before the next winter wheat crop. Corn, for example, develops an environment favorable for seed germination in the fall, the period when goatgrass usually germinates. Barley and oats also encourage fall germination of the weed.

Growers have a couple of options for getting rid of goatgrass that remains after they harvest their summer-annual crop. They can spray herbicides in November, or early April the following year, or they can uproot the goatgrass by cultivation.

"Producers should be able to reduce the number of goatgrass weeds by up to 80 percent by planting two summer crops before the next winter wheat crop. We are confident that an additional summer crop in this rotation would reduce weed populations even further," says Anderson.

Ogg says his work in ARS' Nonirrigated Agricultural Weed Science Research Unit indicates that three annual crops are needed where wheat fields have severe goatgrass infestations. He recommends growers plant spring wheat, barley, canola, peas, or lentils in rotation with winter wheat.

Crop rotations benefit farmers in other ways too, holding insect populations and soil diseases to tolerable levels and helping to maintain soil fertility.—By **Dennis Senft,** ARS.

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Seasonal Seesaw in Body Composition

Older women can expect seasonal changes in their fat and lean mass, even if they maintain the same weight and activity levels throughout the year. That's what researchers at the USDA Human Nutrition Research Center on Aging at Tufts found when they measured 125 women in a whole-body scanner during a year-long study.

The women—all past menopause—had a significant increase in muscle and bone mass after the summer/fall period and a significant decrease after the winter/spring period, says physician Bess Dawson-Hughes. Conversely, their fat tissue decreased after the summer/fall period and increased after the winter/spring period, everywhere but in the arms. But their weight didn't change during the year, she says. Nor could she account for the changes in body composition from any differences in physical activity.

"The more active women had the same degree of fluctuation in lean and fat tissue as the less active," says Dawson-Hughes. "It does not appear to be related to physical activity."

She suspects it may be due to seasonal changes in the activity of the brain's hypothalamus-pituitary area. "This area regulates several major hormones that have been shown to have annual rhythms in one animal species or another," she says. These include growth and sex hormones as well as hormones secreted by the adrenal and thyroid glands.

She says the seasonal fluctuations in fat and lean mass occurred in the women's arm and legs as well as their trunks. By the end of the year, the women had a net loss of lean tissue in their legs and a net gain in their trunks.

"This puts into numbers what we have all seen as people age," she says. "The trunk appears to get thicker and the legs appear to get thinner and thinner. Although we can expect lean tissue to fluctuate with time of year, I think that people can stem a net loss with more exercise."—By **Judy McBride,** ARS.

Bess Dawson-Hughes is at the USDA/ARS Human Nutrition Research Center on Aging at Tufts, 711 Washington St., Boston, MA 02111. Phone (617) 556-3066, fax number (617) 556-3344. ◆

Off-Season Cover Crops Control Erosion

Normally, farmers plant winter-hardy cover crops to keep wind from blowing soil away, but these crops usually have to be killed with herbicides in the spring.

So James D. Bilbro, Jr., an ARS agronomist in Big Spring, Texas, asks why use herbicides when you could let frost do the work?

He is testing whether warm-weather crops such as forage sorghum, planted in late summer or fall, can serve the same erosion prevention purpose.

So far, he's found that frost-killed cover crops provide as much soil- and moisture-saving mulch as live ones. If established by early September, these crops will be dead by December but still provide at least the 60-percent ground cover needed to eliminate wind erosion.

Of the 16 crops being tested, forage sorghum is one of the most promising.

Because the fall-planted, warm-weather plants live such a short time, they leave more soil moisture in the spring than if winter-hardy crops had been planted. The technique is being developed in the Texas High Plains, but it may be useful wherever wind erosion and drought are problems.

Wind damaged 4.3 million acres of cropland and rangeland in the 10-state Great Plains area during the November 1991 to May 1992 wind erosion season, according to the USDA Soil Conservation Service.

More than 16 million acres were reported to be in a condition in which topsoil would blow.

The planting of cover crops and other conservation practices—in addition to easing drought—resulted in far fewer eroded acres than the 8.2 million reported damaged in the 1990-1991 season.—By **Don Comis,** ARS.

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(K2681-13)

Gene Predicts Lamb Leanness

Leaner and larger lamb chops, cutlets, and roasts may show up on your dinner table in the next decade, thanks in part to ARS research aimed at breeding lambs with less fat.

The key is finding an easy way to predict whether lambs will be lean or fat, says ARS geneticist Gary D. Snowder. "One way to do that is with a genetic marker—a specific gene that correlates with how lean an animal will likely be."

The marker could help researchers select genetically superior animals for future breeding. It could also help farmers tailor their lambs' diets to keep them fit and trim.

Meat from such animals would likely appeal to health-conscious consumers who want to eat less fat.

Snowder, who is at the U.S. Sheep Experiment Station near Dubois, Idaho, and colleagues at Utah State University found that two different genes important in fat metabolism—adipocyte P2 (AP2) and lipoprotein lipase (LPL)—may be useful markers to identify leaner lambs.

Earlier, researchers elsewhere discovered both genes in humans, cattle, mice, and birds. Snowder's study is the first to link them with specific characteristics used to determine fatness in lambs.

The researchers analyzed genetic material (RNA) found in fat samples from 18 slaughtered lambs. They also took 10 measurements of each carcass, such as weight, body wall thickness, and kidney fat.

Measuring RNA levels allows scientists to determine the number of times that gene is copied, or expressed, within the cell. Every gene codes for a particular protein. The more often that gene is expressed, the higher the level of that protein.

"We found that fatter lambs tended to have higher levels of AP2 gene expression, yet lower levels of LPL gene expression," says Snowder.

The genes are just two of seven that Snowder plans to test. Once he finds which genes are the most accurate predictors, Snowder says a simple blood test, taken as early as birth, might be all it takes to pinpoint fat-prone lambs.

In the long term, scientists may be able to use the markers to select animals for breeding future generations of leaner lambs.—By **Julie Corliss,** ARS.

Gary D. Snowder is in the USDA-ARS Range Sheep Production Efficiency Research Unit, U.S. Sheep Experiment Station, Box 2010, Dubois, ID, 83423. Phone (208) 374-5306, fax number (208) 374-5582. ◆

Vitamin A's Relative Benefits

Beta carotene's reputation for preventing cancer may be due partly to the body's ability to convert it into retinoic acid.

This vitamin A relative is being successfully used in cancer treatment programs here and abroad. But it's quite toxic to tissues, so the body breaks it down quickly.

Now, studies of cultured human and animal cells and of ferrets—which metabolize beta carotene much the same as people do—show that intestinal cells themselves convert a small portion of beta carotene into retinoic acid and other vitamin A-related compounds. These compounds are absorbed by the cells and move into circulation slowly to prevent toxic levels.

The studies also found that lung, liver, kidney, and fat tissues can convert beta carotene into retinoic acid and its relatives. This suggests that we can raise levels of retinoic acid in body fluids or tissues by eating more foods high in beta carotene, which is not toxic even in fairly large doses.

Orange and yellow fruits and vegetables and dark-green, leafy vegetables are rich in beta carotene and other carotenoids.—By **Judy McBride,** ARS.

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Two Nitrogens Better Than One

When the Pilgrims arrived 300 years ago, Native Americans taught them to plant corn and to fertilize it by burying a fish near each hill of corn seeds. As the fish decayed during the season, it nourished the growing corn.

Scientists have conducted much research since then but admit they must still learn more about making nitrogen fertilization a more efficient practice. Concern heightened in recent years as fertilizer prices increased and, more recently, as people realized that excessive or poorly timed applications can result in the fertilizer ending up in water supplies.

Sterling R. Olsen, an ARS soil scientist, has fine-tuned fertilizer applications and increased corn yields up to 40 bushels per acre. His secret? He used two forms of nitrogen—ammonium and nitrate. Growers today typically use one form or the other.

"Applying the two forms at a rate that provides the same amount of available nitrogen as is usually applied in a single form increases the crop's fertilizer use efficiency: The plants assimilate more. Such application also reduces the danger that some nitrogen will end up in water supplies," says Olsen in Fort Collins, Colorado.

Nitrogen fertilizers are either organic (manure and plant residues), or inorganic (commercial fertilizers). The organic forms must decompose before plants can use them. Inorganic forms are applied as nitrates and ammonium salts, or a combination of the two. Plants take up both nitrates and ammonium salts, but they must convert the nitrate to ammonia before it can be used for growth.

Olsen found the most efficient nitrogen fertilizer combination to be the two forms of nitrogen—nitrate and ammonium—applied in equal amounts. A commercially available nitrification inhibitor, called N-Serve, helped keep the ammonium form of nitrogen from being converted to nitrate before corn roots could absorb it. Top corn yields were 221 bushels per acre.

Olsen, now retired, conducted these tests in cooperation with Colorado State University on irrigated corn growing on calcareous soils low in organic matter.—By **Dennis Senft,** ARS.

Sterling R. Olsen was formerly with the USDA-ARS Water Management Research Unit, Fort Collins, CO 80523. His home address is 1909 Crestmore Place, Fort Collins, CO 80521. Home phone (303) 482-7651. ◆

Maternal Gene Means Life or Death for Flour Beetle

Researchers at Manhattan, Kansas, have found that the red flour beetle—a serious pest in stored grain—harbors a natural population-control gene.

When the female parent beetle has the gene, those of her offspring that do not inherit it die. But if only the male parent possesses and passes on the gene, all offspring survive, regardless of whether the gene is inherited.

And if both parents have the gene, the offspring will live—as long as they inherit the gene from one parent or the other.

"This is a unique system that we call maternal self-selection. That is, the mother is actually determining, with her gene, which of her offspring will live," says Richard W. Beeman, an entomologist at the U.S. Grain Marketing Research Laboratory in Manhattan, Kansas.

Adding to the mystery is the fact that the crucial gene appears to have no real function of its own. However, it may be the first detected member of a type of maternally acting selfish genes that exist, more or less, to perpetuate themselves and, possibly, to aid evolution. Such genes may occur throughout plant and animal species.

The trait is found in flour beetle strains scattered throughout the world, Beeman says. However, it was discovered only when female hybrids between flour beetle strains collected in Singapore and the United States were mated with male beetles from the U.S. strain. The scientists noted that some of the progeny from the crosses lived, while others did not.

Further genetic tests revealed that the beetles from Singapore harbored the lethal gene. Additional tests on the gene are under way.

Beeman and colleagues also found a similar maternal self-selection system in a species known as the confused flour beetle.

Entomologists are hopeful that the gene can somehow be used as a weapon for biological control of insect pests, perhaps by transferring the crucial gene into other insects via genetic engineering.

Beeman's research is being conducted in collaboration with Rob Denell of the Kansas State University Division of Biology.—By **Marcie Gerrietts**, ARS.

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